Original Article

N^6-methyladenosine reader *IGF2BP3* as a prognostic Biomarker contribute to malignant progression of glioma

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Background: Glioblastoma (GBM) is a highly aggressive cancer having a dismal prognosis. N^6-methyladenosine (m^6A) is closely related to GBM progression. The significance of m^6A modifications depends on the m^6A readers, whose functions in glioma progression are largely unknown. This study sought to investigate the expression of the m^6A related gene in glioma and its effect on the malignant progression of glioma.

Methods: The expression differences between low-grade gliomas (LGGs) and high-grade gliomas (HGGs), and among 19 m6A-related genes were analyzed by The Cancer Genome Atlas (TCGA). Survival probability was analyzed in terms of the high or low expression of insulin growth factor-2 binding protein 3 (*IGF2BP3*) in the TCGA data set. The clinicopathological data of 40 patients with glioma were analyzed retrospectively, and the expression of *IGF2BP3* in the tumor tissues was analyzed by immunohistochemistry (IHC). Lentiviral vectors harboring short-hairpin RNA (shRNA) were used to knock down *IGF2BP3* in the glioma cell lines U87 and U251, and the results were verified by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and western blot. The effects of IGF2BP3 on the proliferation, invasion, and tumorigenicity of the glioma cells were verified by Cell Counting Kit-8 (CCK-8), transwell invasion, and subcutaneous tumorigenesis experiments in nude mice. The cell cycle phases were measured by flow cytometry.

Results: The sequencing of TCGA data identified *IGF2BP3* as the most significantly altered m^6A-related gene. Patients with high *IGF2BP3* expression had a significantly reduced survival probability ($P<0.001$) compared to those with low *IGF2BP3* expression. *IGF2BP3* was more upregulated in the HGGs than the LGGs. The downregulation of *IGF2BP3* inhibited the proliferation, migration, and invasiveness of the glioma cells, and xenograft tumor growth in the mice. According to TCGA data, *IGF2BP3* was closely related to cell cycle regulators, such as cyclin-dependent kinase 1 (*CDK1*) and cell-division cycle protein 20 homologue (*CDC20*). Further, the knockdown of *IGF2BP3* affected the expression of *CDK1* and the cell cycle process.

Conclusions: *IGF2BP3* expression in glioma is positively correlated with tumor grade and enhanced glioma cell proliferation, invasion, and tumorigenicity. *IGF2BP3* knockdown decreased the expression of *CDK1* and the cell cycle process. The current study showed that *IGF2BP3* may serve as a biomarker of prognosis and a therapeutic target in glioma.

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Keywords: N^6-methyladenosine (m^{6}A); insulin growth factor-2 binding protein 3 (*IGF2BP3*); glioma; proliferation; invasion

Submitted Feb 15, 2023. Accepted for publication Apr 23, 2023. Published online Apr 25, 2023.
doi: 10.21037/tcr-23-449
View this article at: https://dx.doi.org/10.21037/tcr-23-449

Introduction

Glioma is one of the most common primary adult brain tumors, and accounts for >70% of all brain malignancies, among which glioblastoma (GBM) has the highest malignant potential (1). The prognosis of GBM patients is extremely poor (2). Various treatments are available for GBM; however, the median survival time of affected patients is only about 14.6 months (3). The poor prognosis of GBM patients may be due to the highly heterogeneous nature of GBM and the undefined molecular mechanisms underpinning tumorigenesis and tumor development (4). Our understanding of the biological characteristics of GBM is increasing, and new therapeutic targets have been gradually discovered. However, diverse molecular features of GBM are major obstacles to accurately predicting survival and evaluating the efficacy of chemotherapy or radiotherapy.

Recent research has shown that RNA modifications, especially N^6-methyladenosine (m^{6}A) modifications, are closely associated with malignant progression in cancer (5).

Highlight box

Key findings

- *IGF2BP3* expression in glioma is positively correlated with tumor grade and enhances glioma cell proliferation, invasion, and tumorigenicity.

What is known and what is new?

- Glioma is the most common and aggressive malignant tumor in the central nervous system, and patients with glioma have a poor prognosis. m6A is closely related to GBM progression, and *IGF2BP3*, as an m6A reader, plays a significant role in m6A modifications.
- This study provided novel insights into the effect of *IGF2BP3* on the malignant progression of glioma and its prognostic value in glioma.

What is the implication, and what should change now?

- *IGF2BP3*, as an m6A reader, may serve as a new biomarker of prognosis and a potential therapeutic target for the treatment of glioma.

RNA m^{6}A modification proteins regulate tumorigenesis, tumor development, metastasis, and cancer cell sensitivity to anti-tumor treatments (6,7). Targeting m^{6}A modification regulators might be a potential and promising therapeutic strategy for cancer treatment. The m^{6}A modification is reversible and dynamic, and methyltransferases (writers), demethylases (erasers), and binding proteins (readers) regulate m6A methylation (8). Moreover, the fate of m^{6}A-modified messenger RNAs (mRNAs) depends on m^{6}A readers. Currently, little is known about the effect of m^{6}A reader proteins on glioma progression.

A previous study found that m^{6}A reader insulin growth factor-2 binding protein 3 (*IGF2BP3*) expression is associated with patient survival (9); however, the associated mechanism remains unclear. *IGF2BP3* has also been reported to exhibit oncogenic effects as an RNA-binding protein (RBP) in several tumor types. It plays important role in cancer metabolism, immunity, angiogenesis, stemness, and differentiation. The present study analyzed *IGF2BP3* expression in gliomas of diverse grades, determined the effects of *IGF2BP3* on the biological functions of the glioma cells, and explored the mechanism regulating the biological functions of gliomas. We present the following article in accordance with the MDAR and ARRIVE reporting checklists (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-449/rc).

Methods

Public data sets and bioinformatics analysis

To analyze gene expression alterations in glioma, RNA-sequencing data were downloaded from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/). The expression differences between low-grade gliomas (LGGs) and high-grade gliomas (HGGs), and among the 19 m^{6}A-related genes were then analyzed. Based on the survival analysis cut-off values, the patients were divided into the low- and high-*IGF2BP3* groups. Survival package was used to test the proportional risk hypothesis and fitted survival regression. The results were visualized using

survminer package and ggplot2 package. The limma R package was used to analyze differential gene expression, with a $|\log_2$fold change (FC)$|$ >1 and an adjusted P<0.05 indicating differential mRNA expression. DAVID was applied to determine the Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and Cytoscape was used for visualization. The hub genes were assessed by the Matthews correlation coefficient (MCC) method and identified by the Cytoscape plugin cytoHubba.

Patients and tissue samples

In total, 40 patients with glioma who underwent neurosurgery at The Second Affiliated Hospital of Soochow University were selected and divided into the LGG group [World Health Organization (WHO) grade I–II] and the HGG group (WHO grade III–IV). The patients had a mean age of 58 years and had not been treated with radiation therapy or chemotherapy before surgery. Immunohistochemistry (IHC) analyses were performed on the tumor paraffin sections. Each patient signed the informed consent form. The current study was approved by the Ethics Committee of The Second Affiliated Hospital of Soochow University (No. JD-HG-2022-52). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

IHC

For the IHC analyses, formalin-fixed paraffin-embedded sections underwent xylene deparaffinization and hydration, followed by subsequent antigen retrieval by microwave-heating in sodium citrate buffer. After incubation overnight with the primary antibodies *IGF2BP3* and *Ki-67* (Abcam, UK; ab179807), the slides were then incubated with anti-rabbit horseradish peroxidase-linked secondary antibodies (Invitrogen, USA; C31460100). The samples were then incubated with 3,3-diaminobenzidine and counterstained with hematoxylin and then examined under a microscope in a blinded manner by two pathologists.

Cell culture and transfection

The human glioma U87 and U251 cells were provided by the National Collection of Authenticated Cell Cultures (China). The short-hairpin RNAs (shRNAs) for human *IGF2BP3* (sh1-sequence, CGGTGAATGAACTTCAGAATT; sh2-sequence, GCAGTTGTAAATGTAACCTAT) were synthesized by Genechem (China) and packaged into pLKO.1 (lentiviral vector) to generate pLKO.1-sh*IGF2BP3* silencing constructs, with the empty vector serving as the negative control (NC). Puromycin was used to select the cells with stable transduction, and transduction efficiency was reflected by green fluorescent protein expression. The transfection was performed as directed by the manufacturer. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and immunoblot were conducted to confirm the knockdown of *IGF2BP3*.

QRT-PCR

The total RNA was extracted by TRIzol reagent (Invitrogen) and was then reverse transcribed into complementary DNA using the PrimeScript™ RT reagent kit (Takara, Japan). The expression of mRNA was detected by the SYBR Premix Ex Taq™ kit (Takara), using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for normalization. The sequences of the primers were as follows (5' to 3'): *IGF2BP3*, forward TCACTTCTATGCTTGCCAGGTTGC and reverse CCTTCTGTTGTTGGTGCTGCTTTAC; *GAPDH*, forward TGACATCAAGAAGGTGGTGAAGCAG and reverse GTGTCGCTGTTGAAGTCAGAGGAG.

Immunoblot

Radioimmunoprecipitation assay buffer (Beyotime, China) was used to extract the total protein from the glioma cells. After protein quantitation, the protein samples were resolved by 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis, and then electro-transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). Next, the PVDF membranes were blocked with Tris-buffered saline containing 5% non-fat milk powder at room temperature for 1 h and then incubated overnight with anti-*IGF2BP3* (ProteinTech Group, China; 14642-1-AP), anti-cyclin-dependent kinase 1 (anti-*CDK1*) (ProteinTech Group; 10762-1-AP), and anti-actin (ProteinTech Group; 66009-1-Ig), respectively. Next, secondary antibodies were added at ambient temperature for 1 h and, immunoblots were detected on an imaging system (BioRad, USA).

Cell Counting Kit-8 (CCK-8) assays

Cell viability was measured using the CCK-8 (Dojindo,

Japan) in accordance with the manufacturer's instructions. In brief, 2×10^3 cells were seeded into 96-well plates and incubated for 0, 24, 48, 72, and 96 h. The optical density values were measured at 450 nm after incubation with CCK-8 solution for 1 h.

Clone formation assays

The cells were incubated at 500 cells per 6-well plate at 37 ℃ for 14 days. After washing with phosphate-buffered saline (PBS), fixation was carried out with 2% paraformaldehyde, followed by crystal violet staining and imaging to record the number of generated cell clones. The clone formation rate was calculated as follows: clone formation rate (%) = the number of clones/500×100.

Transwell assays

A 24-well transwell system (Corning, USA) was used to assess cell invasion and migration. Approximately 2×10^4 cells were seeded into the superior compartment, with 850 μL of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in the lower compartment. After being incubated at 37 ℃ with 5% carbon dioxide for 24 h, the cells was fixed with 2% paraformaldehyde and stained with crystal violet. After wiping the cells in the above chamber with a cotton swab, the cells remaining in the lower chamber were counted.

Wound-healing assays

A total of 10^6 cells were planted in low-serum medium (DMEM with 1% FBS). At 90–100% confluency, a line was generated with a 200-μL pipet tip, and the cells were then washed with PBS. The cells were then further incubated in serum-free medium for 24 h. The scratch areas were imaged and compared under a microscope.

Subcutaneous nude mouse xenograft model

The 5-week-old female Balb/c-nu mice from Vital River (China) were randomly allocated to the U87-NC and U87-sh*IGF2BP3* groups (n=5 per group, according to the minimum number of samples required to achieve biological statistical significance; the body weight of mice: 14.70±0.24 g. Next, 10^6 U87-NC or U87-sh*IGF2BP3* cells were injected subcutaneously into the right axilla of each nude mouse. After feeding at our facility for 21 days (on a 12-h light/dark cycle, with free access to drink and food), the mice were anesthetized with isoflurane and sacrificed by cervical dislocation. The subcutaneously transplanted tumor was obtained, weighed, and photographed for analysis. The maximum volume of each subcutaneously transplanted tumor volume was not more than 1,000 mm^3. Next, the tumors were prepared as paraffin sections. The experiments involving animals were approved by the Animal Ethics Committee of Soochow University (No. 20210708A02), all animal work was conducted in compliance with national guidelines for care and human use of animals. A protocol was prepared without registration before the study.

Cell cycle analysis

After being added to 75% ice-cold ethanol, 2×10^5 cells were incubated at 4 ℃ overnight. Next, 1 mL of DNA staining solution and 10 μL of permeabilization solution (BD, USA) were added to the samples, which were then analyzed by flow cytometry.

Statistical analysis

The assays were carried out 3 times, and the data analysis was conducted using GraphPad Prism 9.0.0. The data are presented as the mean ± standard deviation and were compared using the *t*-test. A P value <0.05 indicated statistical significance.

Results

IGF2BP3 was the most significantly altered m^{6}A-related gene

An analysis of the differences in gene expression between the LGGs and HGGs in TCGA data identified 6,595 differentially expressed genes (DEGs) with a ($|\log_2 FC| >1$ and an adjusted P value <0.05) (*Figure 1A*). In addition, an analysis of the expression differences in the 19 m^{6}A-associated genes in glioma revealed that *IGF2BP3* was the most significantly altered m^{6}A-related gene (*Figure 1B*). In a subsequent analysis, we found that patients with high *IGF2BP3* expression had a significantly reduced survival probability (P<0.001) compared to those with low *IGF2BP3* expression (*Figure 1C*).

IGF2BP3 expression level was correlated with glioma grade

The clinicopathological data of 40 patients with glioma

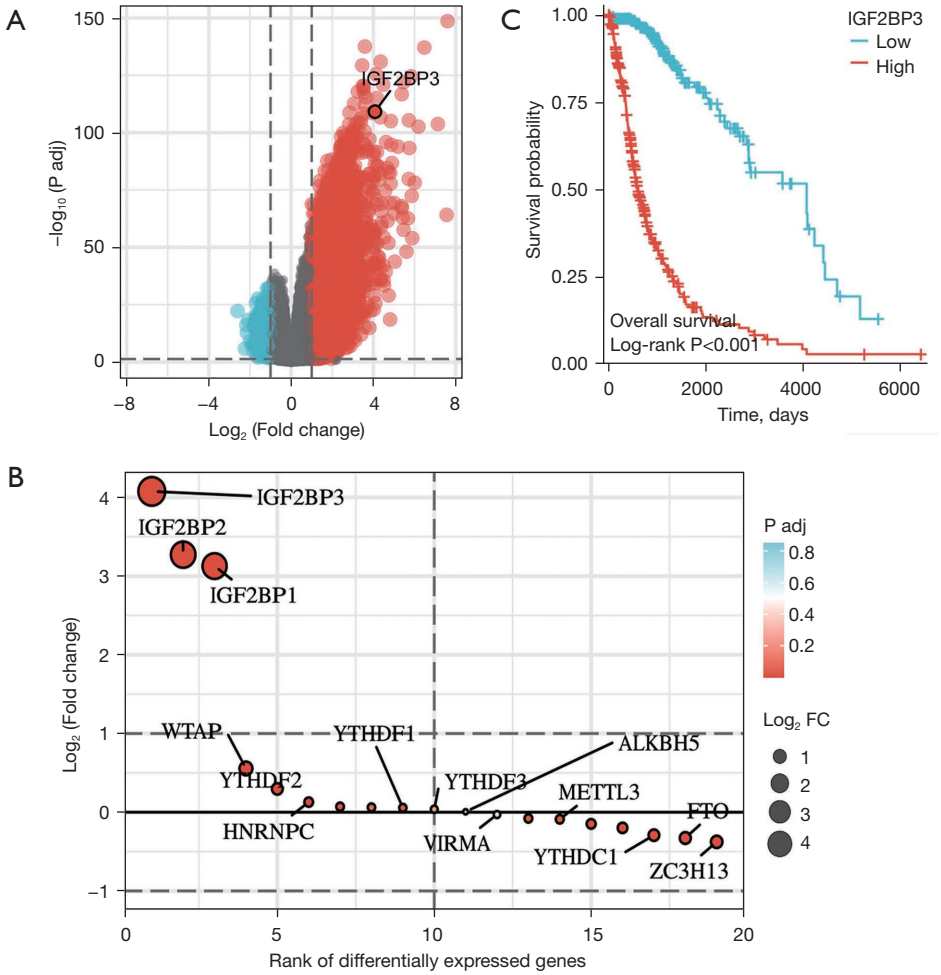

Figure 1 Expression of m6A-related genes in gliomas. (A) By analyzing the gene expression differences of LGGs and HGGs in TCGA, a total of 6,595 DEGs were identified (|log2FC| >1, adjusted P<0.05). (B) The expression differences of the 19 m^{6}A-related genes in gliomas were analyzed, and *IGF2BP3* was the most significantly altered m^{6}A-related gene. (C) Survival probability was analyzed in terms of the high (red) or low (blue) expression of *IGF2BP3* in TCGA data set. *IGF2BP3*, insulin growth factor-2 binding protein 3; FC, fold change; m^{6}A, N^6-methyladenosine; LGGs, low-grade gliomas; HGGs, high-grade gliomas; TCGA, The Cancer Genome Atlas; DEGs, differentially expressed genes.

(20 low-grade and 20 high-grade cases) were analyzed retrospectively. IHC staining of the paired peritumoral and tumor tissue samples showed that *IGF2BP3* expression was very low in the peritumoral tissues (*Figure 2A*). Compared to the LGG tissues (*Figure 2B*), *IGF2BP3* was significantly upregulated in the GBM specimens (*Figure 2C*). The IHC statistical analysis results are shown in *Figure 2D*.

IGF2BP3 silencing inhibited glioma proliferation in vitro

To assess the effect of *IGF2BP3* on glioma cell proliferation, the U87 and U251 cells were transfected with *IGF2BP3* shRNAs. Both the shRNAs (sh1/2) significantly decreased the *IGF2BP3* mRNA and protein levels (*Figure 3A*). CCK-8 assays were used to examine the effect of *IGF2BP3* on

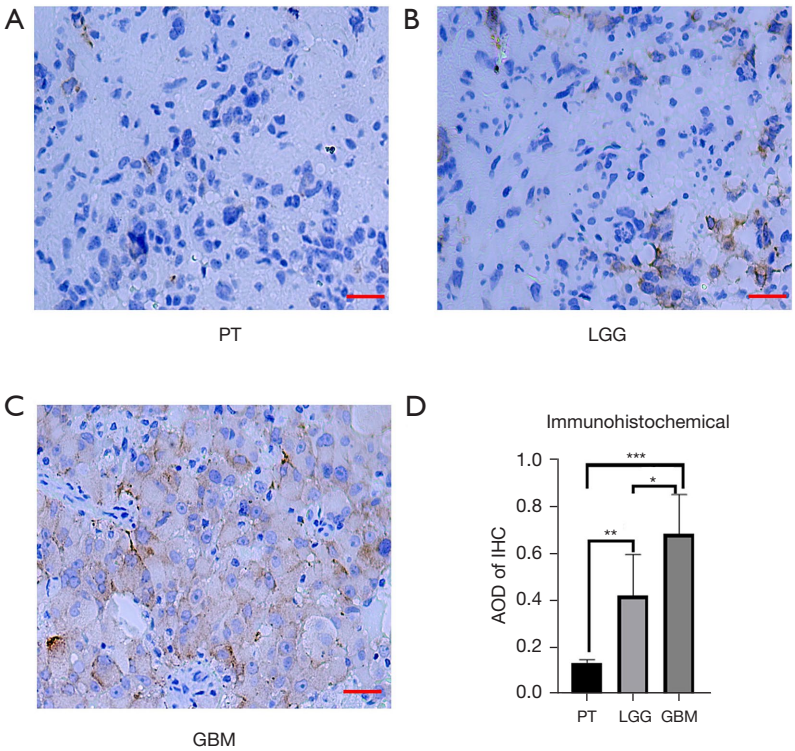

Figure 2 The expression of IGF2BP3 was associated with the histological malignancy of human gliomas. (A) A representative IHC image showing very low *IGF2BP3* expression in PT tissues. (B) A representative IHC image showing low *IGF2BP3* expression in LGG. (C) A representative IHC image showing very high *IGF2BP3* expression in GBM tissues. (D) The expression levels of the *IGF2BP3* proteins in each sample. *, P<0.05; **, P<0.01; ***, P<0.001. Magnification ×200; scale bar =20 μm. PT, peritumoral tissues; LGG, low-grade glioma; GBM, glioblastoma; AOD, average optical density; IHC, immunohistochemistry; *IGF2BP3*, insulin growth factor-2 binding protein 3.

glioma cell proliferation. In this study, *IGF2BP3* silencing significantly inhibited cell proliferation in comparison to the control cells (*Figure 3B*). In addition, *IGF2BP3* silencing reduced the colony formation ability of the U87 and U251 cells (*Figure 3C*). Thus, *IGF2BP3* knockdown repressed glioma cell proliferation.

IGF2BP3 knockdown significantly repressed glioma cell invasion and migration in vitro

Wound-healing assays were performed to determine the effect of *IGF2BP3* on glioma cell migration. As *Figure 4A* shows, *IGF2BP3* silencing significantly reduced the gap closure rate in the GBM cells. In addition, the transwell migration assays showed *IGF2BP3* silencing significantly decreased the migratory (*Figure 4B*) and invasive (*Figure 4C*) abilities of the glioma cells. These findings suggested that *IGF2BP3* was associated with glioma migration and invasion.

IGF2BP3 silencing inhibited the tumorigenic properties of the glioma cells

To evaluate the tumorigenic property of *IGF2BP3 in vivo*, the U87-NC and U87-sh*IGF2BP3* cells were subcutaneously inoculated into the athymic mice (5 mice per cell type). After 21 days, the mice were euthanized, and the xenograft tumors were extracted, imaged, and measured. The U87-NC group had a higher average tumor volume and weight than the U87-sh*IGF2BP3* group (*Figure 5A*). The IHC staining revealed that *IGF2BP3* and *Ki-67* were downregulated in the *IGF2BP3*-deficient tumors (*Figure 5B,5C*). These data suggested that *IGF2BP3* knockdown significantly decreased the tumorigenic

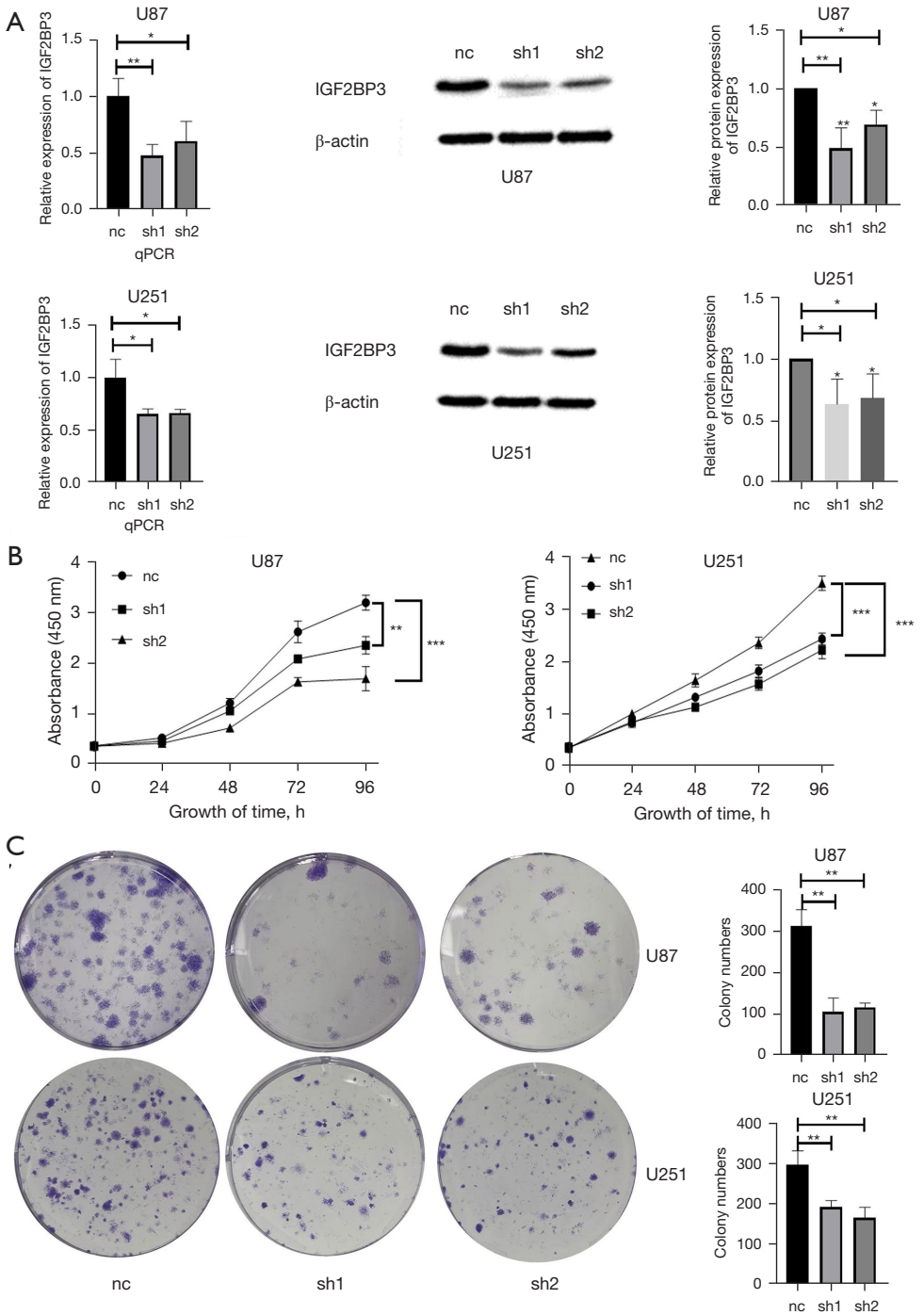

Figure 3 Downregulation of *IGF2BP3* suppressed the proliferation ability of the glioma cells. (A) Knockdown of *IGF2BP3* in the U87 and U251 cells was confirmed by qRT-PCR and western blot analysis. (B) CCK-8 assays were performed to determine cell growth after *IGF2BP3* was knocked down in the U87 and U251 cells. (C) *IGF2BP3* knockdown inhibited the clone formation ability of the U87 and U251 cells (clone formation assay was measured by crystal violet staining assay). *, P<0.05; **, P<0.01; ***, P<0.001. *IGF2BP3*, insulin growth factor-2 binding protein 3; qPCR, quantitative polymerase chain reaction; NC, negative control; sh, short-hairpin; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; CCK-8, Cell Counting Kit-8.

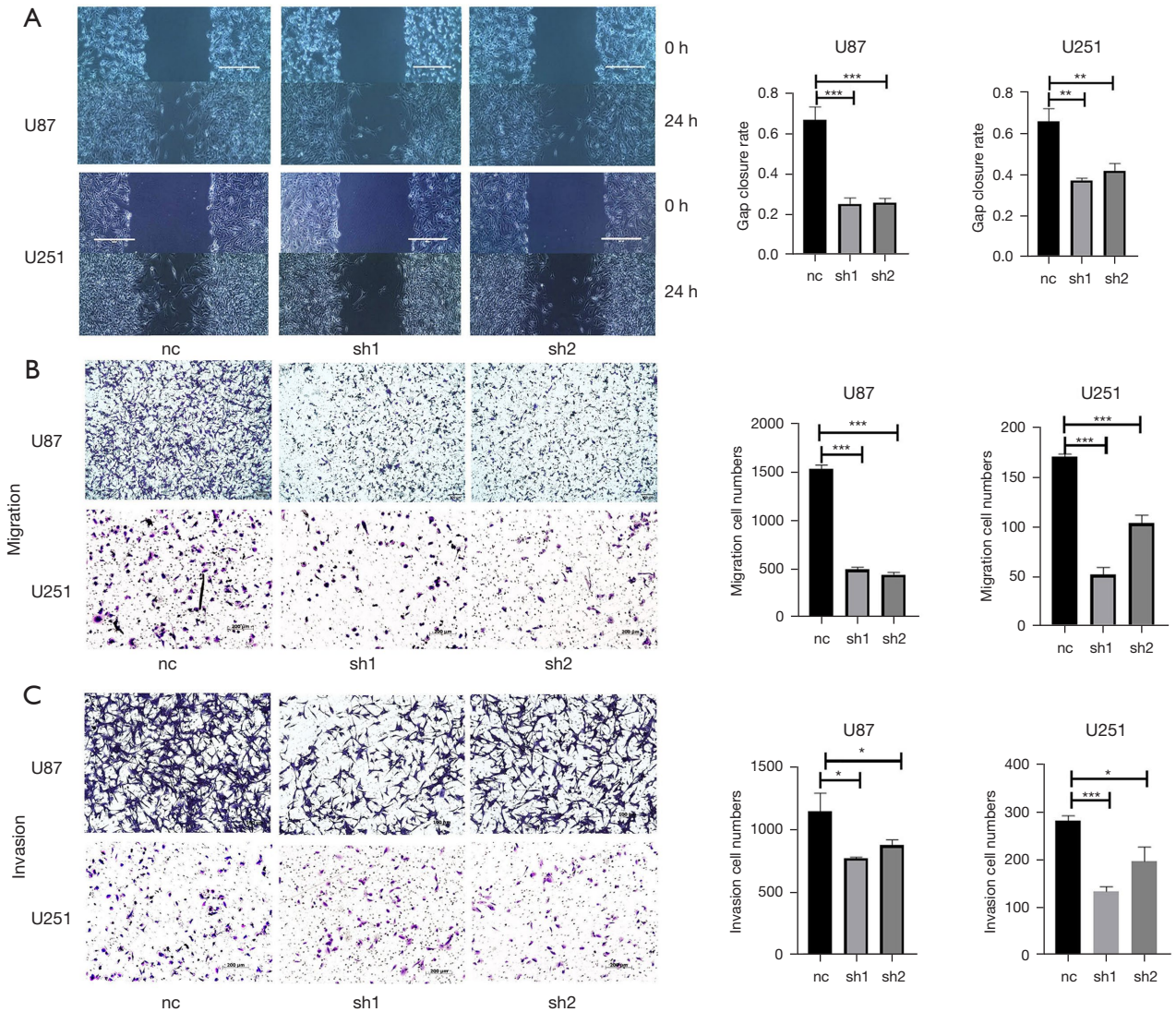

Figure 4 *IGF2BP3* knockdown decreased the migration and invasion abilities of the glioma cells. (A) The cells were scraped and imaged immediately (0 h), and after 24 h; images of the wound gap were taken for analysis (×10). (B) Cell migration assays were carried out in the U87 and U251 cells (×100; cell migration assays were measured by crystal violet staining assay); *IGF2BP3* knockdown by two different shRNAs significantly suppressed cell migration in the U87 and U251 cells. (C) Cell invasion assays were carried out in the U87 and U251 cells, which showed that the knockdown of *IGF2BP3* suppresses the invasion ability of the glioma cells (×100; cell invasion assays were measured by crystal violet staining assay). *, P<0.05; **, P<0.01; ***, P<0.001, *vs.* their respective control. NC, negative control; sh, short-hairpin; *IGF2BP3*, insulin growth factor-2 binding protein 3; shRNAs, short-hairpin RNAs.

properties of the glioma cells *in vivo*.

IGF2BP3-related DEGs in gliomas

To explore the underpinning mechanisms of *IGF2BP3* in glioma carcinogenesis, the RNA-seq data of 448 glioma patients were retrieved from TCGA database. The patients were divided into the low- and high-*IGF2BP3* groups based on survival. In total, 946 DEGs were found to be significantly associated with *IGF2BP3*, among which 567 were upregulated and 379 were downregulated (*Figure 6A*). The results of the GO and KEGG analyses of the DEGs are shown in *Figure 6B*. The *IGF2BP3* silencing in the U87 cells revealed the possible roles of the DEGs. Finally, the

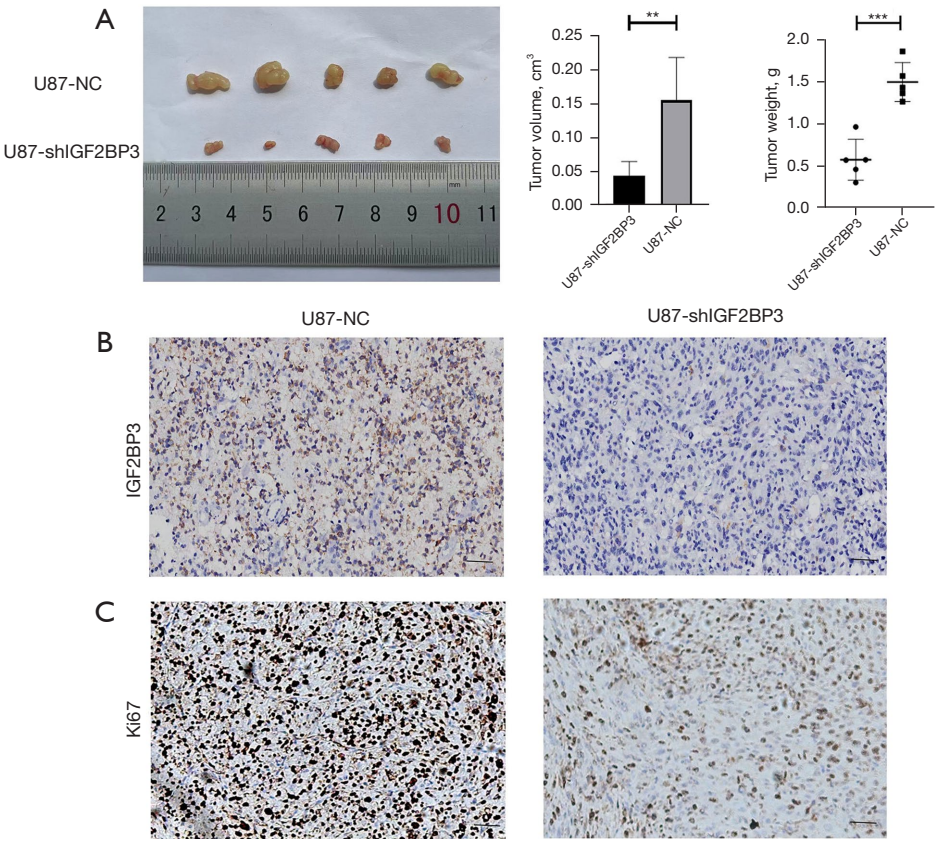

Figure 5 *IGF2BP3* knockdown in U87 cells suppressed tumor growth in the nude mice. (A) Representative images of the xenograft tumors in the nude mice are shown in the left panel, and tumor volume was calculated as follows: (longest diameter) × (shortest diameter)2 × (ω/6). **, P<0.01; ***, P<0.001. (B) Representative images of IHC staining for *IGF2BP3* in tumors excised from xenograft model mice (×200). (C) Representative images of IHC staining for Ki-67 (×200). Scale bar =20 μm. NC, negative control; sh, short-hairpin; *IGF2BP3*, insulin growth factor-2 binding protein 3; IHC, immunohistochemistry.

top 10 hub genes were retrieved with the cytoHubba plugin in Cytoscape (*Figure 6C*).

IGF2BP3 induced glioma cell cycle arrest

According to TCGA data, we found close associations between *IGF2BP3* and the cell cycle regulators, including *CDK1* and cell-division cycle protein 20 homologue (*CDC20*). *CDK1* belongs to the *CDK* family and mainly affects the cell cycle. The western blot analysis demonstrated that *CDK1* expression was significantly reduced after *IGF2BP3* knockdown (*Figure 7A*). Moreover, the flow cytometry results showed that the glioma cells were arrested in the G0/G1 phase after *IGF2BP3* knockdown (*Figure 7B,7C*).

Discussion

RNAs are major information carriers in cells. Several types of RNAs with small molecular weights and large content variations are found in cells. Based on their structure and function, RNAs are classified into coding and non-coding types. Recently, non-coding RNAs and post-transcriptional RNA modifications have become popular areas in cancer research (10). RNA methylation accounts for >60% of all RNA modifications, among which m^6A methylation is the most common and most studied type (11). The m^6A modification is reversible and dynamic and may affect mRNA splicing, stability, and translation efficiency (12,13). Three types of m^6A modulators are involved in m^6A methylation, including writers, erasers, and readers (14).

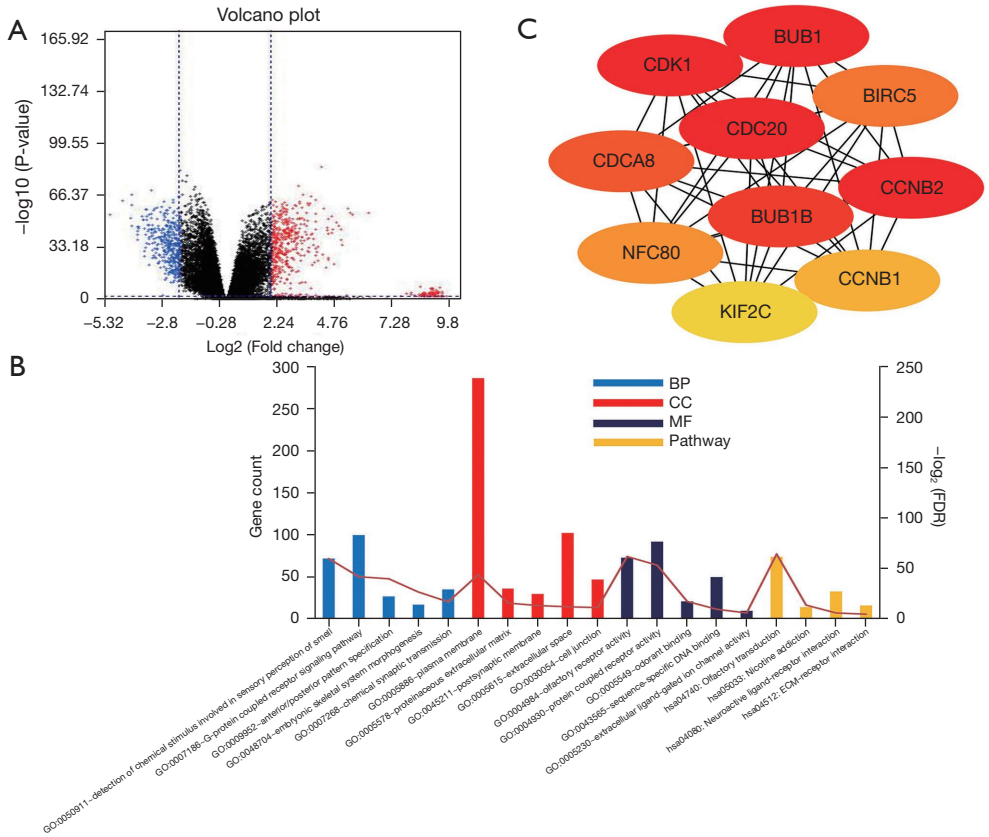

Figure 6 Exploring the target mRNA of *IGF2BP3*. (A) The identified DEGs are shown in the volcano plot. Red represents significantly different up-regulated genes; blue represents significantly different down-regulated genes; black represents genes with no significant difference. (B) The top 5 significant GO and KEGG enrichment terms of the DEGs. (C) The top 10 hub genes were selected through the cytoHubba App in Cytoscape. BP, biological progress; CC, cellular component; MF, molecular function; FDR, false discovery rate; GO, Gene Ontology; mRNA, messenger RNA; *IGF2BP3*, insulin growth factor-2 binding protein 3; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

There is mounting evidence that m^{6}A methylation significantly affects RNA metabolism and regulates the pathogenetic mechanisms of various pathologies, including cancer (15). Wang *et al.* (16) found that three different m^{6}A modification clusters affect the immune microenvironment of esophageal cancer, and their findings provided major insights into the diagnostic and therapeutic approaches. The overexpression of m^{6}A methylation-associated genes plays a critical role in acute myeloid leukemia, breast cancer, nasopharyngeal carcinoma, colorectal cancer, and osteosarcoma (17-21).

GBM is the most common primary intracranial malignancy, and neither surgery nor chemotherapy can improve its prognosis (22). Besides conventional radiotherapy and chemotherapy, novel technologies, such as targeted therapy, immunotherapy, and electric field therapy, are gradually being assessed clinically. However, most cases of malignant glioma show tumor recurrence and progression because existing treatment strategies only target a single key oncogenic pathway or gene mutation in glioma cells. Typically, GBMs are highly heterogeneous, and glioma stem cells (GSCs) switch between different states of molecular subtypes. In recent years, multiple studies in the fields of genomics, epigenetics, and tumor immunology have identified several molecular markers that may be used for precise diagnosis, prognosis assessment, and individualized treatment (23).

Interestingly, the RNA field has emerged as a new frontier in cancer therapy. RNA modifications play a critical role in tumor development. m^{6}A, which is the

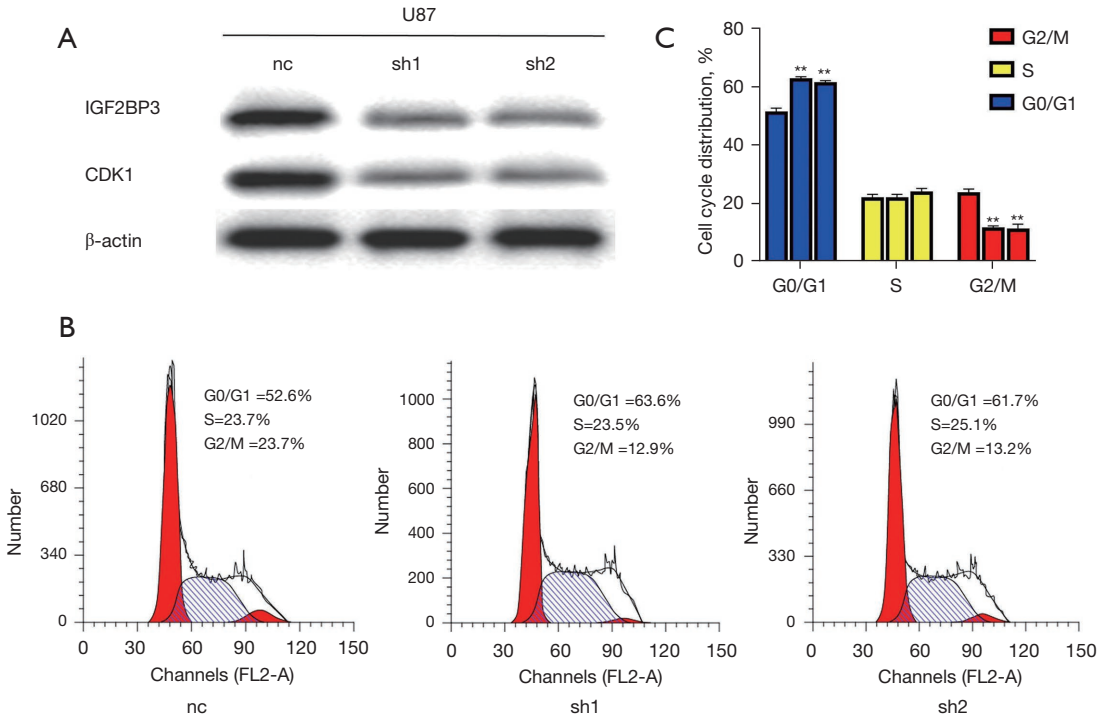

Figure 7 *IGF2BP3* silencing induced cell cycle arrest in the U87 cells. (A) Western blotting revealed that *IGF2BP3* knockdown decreased the protein levels of *CDK1*. (B) FACS analysis results showing the proportion of cells in each phase of the cell cycle. (C) Bar graph showing the results of the number of cells in each phase of the cell cycle. All the experiments were conducted 3 times. The results are presented as the mean ± standard deviation (**, P<0.01). *IGF2BP3*, insulin growth factor-2 binding protein 3; *CDK1*, cyclin-dependent kinase 1; NC, negative control; sh, short-hairpin; FACS, fluorescence-activated cell sorting.

most well-known RNA modification, is closely related to GBM progression and invasiveness. Methyltransferase-like 3 (*METTL3*), an m^{6}A writer, induces GBM growth and progression; specifically, *METTL3* silencing inhibits the growth and self-renewal ability of GSCs (24). Visvanathan *et al.* (25) reported elevated *METTL3* levels in GSCs, which play a crucial role in GSC maintenance and resistance to γ-irradiation. A GO analysis demonstrated *METTL3* was involved in key carcinogenic pathways, including the vascular endothelial growth factor signaling pathway, angiogenesis, tumor metabolism, G protein coupled receptor signaling, and cadherin signaling (26). However, inconsistent findings have been reported for GBM. Notably, Cui *et al.* found that *METTL3* knockdown promotes the proliferative, self-renewal, and tumorigenic abilities of GSCs (27), which suggests *METTL3* serves as a tumor suppressor in GBM. It may be difficult to determine the factors contributing to the controversial role of *METTL3* in GBM. A variety of m^{6}A readers and different cell types and tumor specimens could account for this variation (28).

Like writers, m^{6}A erasers are important in GBM. The fat mass and obesity-associated (*FTO*) gene, which was the first m^{6}A eraser associated with obesity (29), plays an important oncogenic role in GBM. Cui *et al.* (27) indicated that *FTO* knockdown inhibits GSC growth and self-renewal. Similarly, Su *et al.* showed that *FTO* inhibition decreases the self-renewal and carcinogenic abilities of cultured GSCs in a mouse model of GBM (30). Further, the overexpression of the m^{6}A demethylase *ALKBH5* in GSCs increases the self-renewal, proliferation, and tumorigenicity of cells (31).

In addition to the methylases and demethylases, an indispensable protein group involved in m^{6}A are "readers". The significance of m^{6}A modifications in cells depends on m^{6}A readers, as they recognize and interact with methylated modification sites and participate in downstream RNA translation and degradation. Research has shown that even in the case of unchanged m^{6}A levels, abnormal m^{6}A reader expression promotes GBM tumorigenesis (3). The most well-known m^{6}A readers include the YTH domain containing (*YTHDC*) and YTH N^6-methyladenosine

RNA binding protein (*YTHDF*) families, which have a YTH domain (32,33). An additional family of m^{6}A readers (i.e., *IGF2BPs*, including *IGF2BP1–3*) has also been identified (34). IGF2BPs have long been considered oncofetal proteins in numerous cancer tissues and play an oncogenic role (35). Suvasini *et al.* (36) reported that *IGF2BP3* is upregulated in GBM but not significantly in low-grade astrocytoma samples. In addition, *IGF2BP3* increases the proliferative abilities of GBM cells by inducing epithelial-mesenchymal transition (EMT) (37). However, it is unclear whether the above phenomenon involves the m^{6}A reading process.

In the present study, an analysis of the m^{6}A-associated genes in gliomas of TCGA revealed that *IGF2BP3* was the most commonly regulated m^{6}A-associated gene, and the high *IGF2BP3* expression was shown to predict short survival (P<0.001). As stated above, the *IGF2BP3* expression levels were high in GBM, low in LGG, and extremely low in the paratumor adjacent tissues. We also observed that *IGF2BP3* knockdown significantly reduced the proliferative, migratory, and invasive abilities of the glioma cells and suppressed glioma tumor growth. Thus, we can conclude that *IGF2BP3* is important in human glioma progression, but the underpinning mechanisms require further investigation.

Huang *et al.* (34) found that *IGF2BPs* enhance the stability of their mRNA targets in an m^{6}A-dependent fashion. Zhang *et al.* (38) showed that *IGF2BP3* downregulation inhibits acute myeloid leukaemia (*AML*) progression by altering the stability of regulator of chromosome condensation 2 (*RCC2*) mRNA in an m^{6}A-dependent fashion. As an m^{6}A reader protein, *IGF2BP3* exerts major biological effects by recognizing the target genes. Thus, we explored *IGF2BP3* targets in glioma by a bioinformatics analysis based on TCGA data, and identified 567 upregulated and 379 downregulated genes with significant associations with *IGF2BP3*. We also selected the top 10 hub genes that appeared promising for further investigation, many of which were associated with cell cycle progression. A cell cycle analysis by flow cytometry showed that the cell cycle was arrested after *IGF2BP3* silencing. Additionally, we found *IGF2BP3* knockdown decreased the expression of *CDK1*, which is essential for cell cycle regulation.

Abnormal m^{6}A modification is closely related to cancer occurrence, development progression, and cancer metabolism (39). Recently a study has found that the expression of m^{6}A regulators are related to the infiltration of tumor immune cells (40). In multiple tumor types including LGG, *IGF2BP3* expression was positively correlated with the infiltration of various immune cells such as CD4$^+$ T cell, CD8$^+$ T cell, neutrophils, macrophages and dendritic cells (DCs). The *IGF2BP* family plays a pivotal role by recognizing m^{6}A modifications and suppressing RNA degradation. We analyzed the biological function of *IGF2BP3* in glioma and searched for its downstream target genes. The current study highlighted *IGF2BP3* as a therapeutic target in glioma; however, further research needs to be conducted to confirm these findings.

Conclusions

IGF2BP3, which is the most significantly altered m^{6}A-related gene, is correlated with glioma prognosis. *IGF2BP3* expression in glioma is positively correlated with tumor grade and enhances glioma cell proliferation, invasion, and tumorigenicity. *IGF2BP3* knockdown decreases the expression of *CDK1* and the cell cycle process and thus may serve as a potential therapeutic target for glioma.

Acknowledgments

Funding: This study was supported by the Scientific and Technological Development Plan Project of Suzhou (No. SYS2020142), the Suzhou High-Tech Zone Youth Science and Technology Project (No. 2019Q010), and the Advanced Research Foundation of The Second Affiliated Hospital of Soochow University (No. SDFEYJHJ2101).

Footnote

Reporting Checklist: The authors have completed the MDAR and ARRIVE reporting checklists. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-449/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-449/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-449/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-449/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Each patient signed the informed consent form. The current study was approved by the Ethics Committee of The Second Affiliated Hospital of Soochow University (No. JD-HG-2022-52). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The experiments involving animals were approved by the Animal Ethics Committee of Soochow University (No. 20210708A02), all animal work was conducted in compliance with national guidelines for care and human use of animals.

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(English Language Editor: L. Huleatt)

Cite this article as: Zheng X, Li S, Yu J, Dai C, Yan S, Chen G, Sun C. N^6-methyladenosine reader *IGF2BP3* as a prognostic Biomarker contribute to malignant progression of glioma. Transl Cancer Res 2023;12(4):992-1005. doi: 10.21037/tcr-23-449

